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Immune Activation and Decreased Tryptophan in Patients with HIV-1 Infection

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ABSTRACT

We compared tryptophan, neopterin, and interferon-γ (IFN-γ) concentrations in serum and cerebrospinal fluid (CSF) of 22 patients with human immunodeficiency virus type 1 (HIV-1) infection. Tryptophan levels were found to be decreased in CSF and serum of patients whereas neopterin levels in CSF and serum and serum IFN-γ concentrations were increased compared to healthy HIV-1 seronegatives. Tryptophan concentrations correlated negatively to neopterin concentrations, and serum neopterin concentrations correlated positively to IFN-γ concentrations. Thus, decrease of tryptophan levels is associated with chronic immune stimulation in patients with HIV-1 infection. From the data it appears that reduced tryptophan in patients may result from induction of indoleamine (2,3)-dioxygenase by IFN-γ.

INTRODUCTION

PATIENTS WHO ARE INFECTED with human immunodeficiency virus type 1 (HIV-1) frequently present with neurologic and psychiatric symptoms.[1] Impaired tryptophan metabolism and reduced availability of 5-hydroxytryptamine (serotonin) could be involved.[2–4] Reduced concentrations of tryptophan were reported in serum and cerebrospinal fluid (CSF) of patients.[2,3] Decreased tryptophan could result from increased activity of indoleamine (2,3)-dioxygenase (IDO), which is induced by interferon-γ (IFN-γ) and degrades tryptophan to form kynurenine, which then is further metabolized.[5] Increased concentrations of neopterin in patients with HIV-1 infection[6–8] reflect chronic immune stimulation. Large quantities of neopterin are released by human macrophages on stimulation with IFN-γ *in vitro*.[9] Release of neopterin by stimulated macrophages is paralleled by significant degradation of tryptophan.[10] Recently, increased concentrations of IFN-γ were reported in serum of HIV-1 seropositives.[11]

In this study, we compared tryptophan concentrations in serum and CSF of patients with HIV-1 infection to the immune activation markers neopterin and IFN-γ. Neopterin and tryptophan data stem from two earlier

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EXHIBIT B

(4)

studies, which reported decreased tryptophan[3] and increased neopterin[6] levels in serum and CSF of a larger cohort of HIV-1 seropositives.

METHODS

Neopterin and tryptophan levels were measured in serum and CSF samples from 22 patients with established HIV-1 infection (ELISA-positive, confirmed by Western blot). Fourteen patients were asymptomatic, four had persistent generalized lymphadenopathy (LAS), and four had AIDS at time of enrollment (Table 1). One LAS patient was treated with metronidazolum and paramomycinum, and the

TABLE 1. PATIENT CHARACTERISTICS AND LABORATORY RESULTS

Case[a]	*Clinical status*[b]	*Age (yrs)*	*Tryptophan (umole/liter)*		*Neopterin (nmole/liter)*		*IFN-γ (U/liter)*	
			Serum	*CSF*	*Serum*	*CSF*	*Serum*	*CSF*
1	AS	28	45.1	2.36	12.4	2.0	—[c]	—
2	AS	37	36.7	1.68	10.6	1.2	—	—
3	AS	25	30.9	1.76	19.6	3.3	34	206
4	AS	25	28.4	1.78	4.9	1.2	21	189
5	AS	31	37.7	1.79	5.8	1.3	78	137
6	AS	28	24.0	2.60	11.2	2.4	77	135
7	AS	47	25.5	2.42	9.5	2.7	37	140
8	AS	39	23.0	0.15	38.4	27.1	99	189
9	AS	21	28.9	1.02	18.3	9.5	50	53
10	AS	25	30.9	1.88	10.6	10.7	50	78
11	AS	65	25.5	1.63	12.8	7.2	26	166
12	AS	48	19.6	1.58	19.8	16.9	240	128
13	AS	26	41.6	1.21	9.9	12.3	51	123
14	AS	51	26.9	0.57	11.4	10.1	44	95
15	LAS	33	32.8	1.43	13.6	4.5	—	—
16	LAS	27	31.3	0.97	9.8	8.3	20	134
17	LAS	49	27.4	1.24	31.4	4.5	394	255
18	LAS	41	31.3	1.27	12.2	4.8	44	124
19	AIDS	40	41.1	1.38	21.6	8.4	—	—
20	AIDS	37	13.7	1.23	126.6	72.7	321	203
21	AIDS	31	26.4	1.39	30.5	12.4	1055	188
22	AIDS	46	26.4	1.22	37.4	23.2	229	71
Mean		36	29.8	1.48	21.7	11.2	159	143
SD		±11	±7.4	±0.57	±25.3	±15.4	±250	±53
HIV seronegative controls[3,6,11]								
n			14	14	359	19	79	—
Mean			39.7	2.18	5.34	1.60	33	—
SD			±8.8	±0.19	±2.70	±0.60	±15	—

[a] All cases are male homosexuals except case 2 (female blood transfusion recipient), case 22 (female with heterosexual transmission), and case 14 (male intravenous drug user).

[b] AS, Asymptomatic; LAS, lymphadenopathy syndrome. All AIDS patients had at least one episode of *Pneumocystis carinii* pneumonia; case 19 had additional Kaposi's sarcoma.

[c] Not done.

AIDS patients were treated with amphotericinum B_4 ($n = 1$), aciclovirum + pyrimethaminum + calcii folinas + amphotericinum B ($n = 2$), or trimethoprimum/sulfamethoxazolum + flunitrazepanum ($n = 11$). All others were free of therapy at the time of enrollment.[3]

Tryptophan concentrations in serum and CSF were determined by high-pressure liquid chromatography.[3] Neopterin levels in serum and CSF were measured by radioimmunoassay (RIAcid, Fa.Henning-Berlin, Berlin, FRG) as previously described.[6] IFN-γ was measured in 18 serum and 18 CSF samples by radioimmunoassay (Centocor Inc., Malvern, PA). The sensitivity of this test was increased to 18 U/liter by increasing the incubation time as described.[11] The test results were compared to HIV-seronegative healthy controls, who have been examined in our laboratories during the underlying primary studies[3,6] and in an earlier study.[11]

Comparison of grouped data was done by Student's t test and results were confirmed by nonparametric tests (not shown in detail). To test for associations between variables we used Spearman's rank correlation coefficients r_s.

RESULTS

Patients with HIV-1 infection had decreased tryptophan ($p < 0.01$ Student's t test) and increased neopterin concentrations ($p < 0.01$) in serum and CSF compared to HIV-1 seronegative controls (Table 1). Circulating IFN-γ in serum was increased ($p < 0.01$). IFN-γ was also detectable in CSF. Treatment status was not associated with any of the parameters in the group of patients (not shown).

Negative correlations existed between neopterin and tryptophan concentrations in both serum ($r_s = -0.42$, $p = 0.05$; Fig. 1) and CSF ($r_s = -0.66$, $p < 0.01$). Serum IFN-γ correlated positively with serum neopterin ($r_s = 0.65$, $p < 0.01$) and negatively but not significantly with serum tryptophan levels ($r_s = -0.39$, n.s.). CSF concentrations of IFN-γ did neither correlate with neopterin nor with tryptophan concentrations in the CSF ($r_s = -0.22$ and 0.20; n.s.).

To explore a possible statistical bias caused by the four AIDS patients, we reevaluated the correlation analyses considering exclusively asymptomatic individuals and patients with lymphadenopathy syndrome. However, the results of the statistics and the interpretation of the data did not change.

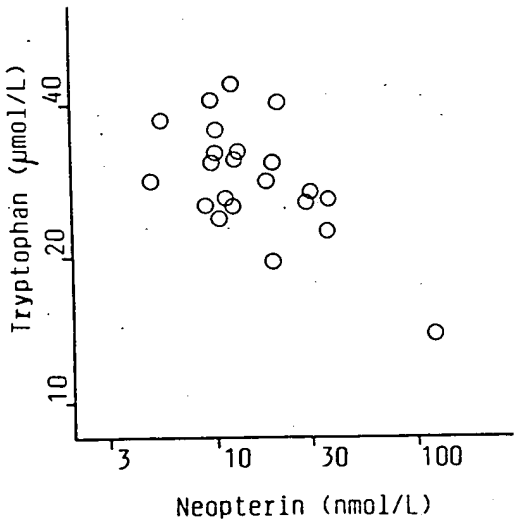

FIG. 1. Correlation between serum neopterin and tryptophan levels in patients with HIV-1 infection (note the double-logarithmic scale). The data suggest a reciprocal relationship, which is significant ($\text{tryptophan}^{-1} = 0.0285 + 3.41 \times 10^{-4} \times \text{neopterin}$; $r = 0.802$, $p < 0.0001$).

DISCUSSION

HIV-1 infection is associated with increased neopterin[6–9] and decreased tryptophan[2,3] concentrations in serum and CSF. Increased levels of circulating IFN-γ have also been reported.[11] The results of the present evaluation confirm these earlier findings. The three variables correlated to a variable degree with the severity of symptoms in the patients. However, the number of samples in the groups with different clinical presentation is too small for meaningful statistical evaluation.

In our patients with HIV-1 infection, we found a positive correlation between serum IFN-γ and neopterin concentrations. In addition, we found that serum tryptophan levels correlate negatively to neopterin. The data agree with the hypothesis that endogenous release of IFN-γ may be the reason for these metabolic changes in patients. Decreased tryptophan appears to be associated with chronic immune activation and may result from increased activity of IDO. IDO and release of neopterin can be induced by IFN-γ.[5,9,10]

The correlation between serum tryptophan and IFN-γ was negative, but was just below statistical significance. The small number of patients in this study could be the reason for the lack of significance. Further studies may help to clarify the possible presence of a correlation between tryptophan and IFN-γ data. Obviously, other cytokines such as IFN-α and tumor necrosis factor-α (TNF-α) can modulate IFN action and hence contribute to additional changes in neopterin and tryptophan concentrations.[9,12] In addition, IFN-γ may bind to receptors on cells,[13] and so free IFN-γ would not reflect its biological activity. This may explain in part why the direct correlation between neopterin and tryptophan was better than that to IFN-γ.

Also CSF neopterin and tryptophan concentrations correlated negatively and IFN-γ could be detected in CSF. Mean IFN-γ levels were similar in serum and CSF samples but many patients had CSF levels higher than those in serum. However, the levels showed only small variation. No correlation existed between CSF IFN-γ and neopterin or tryptophan concentrations in the CSF. Unfortunately, no CSF IFN-γ reference levels of healthy controls were available. Further studies are needed to demonstrate a possible relevance of intrathecal production of IFN-γ in patients with HIV-1 infection and the reliability of the test used in CSF samples. However, intrathecal production of the immune activation markers β_2-microglobulin and neopterin has already been demonstrated in patients with HIV-1 infection.[6,8]

The relevance of IDO activation in HIV-1 seropositive patients is further supported by earlier data showing increased levels of tryptophan metabolites such as kynurenine in serum[2] and quinolinic acid in the CSF,[14] confirming that decreased tryptophan is not simply due to reduced dietary intake.[3,15]

Notably, disturbances of tryptophan metabolism are seen in early stages of HIV-1 infection and independently from neurologic or psychiatric abnormalities of patients. However, a significant correlation between decreased tryptophan and neuropsychological disturbances such as dementia and polyneuropathy in patients with HIV-1 infection was demonstrated in earlier studies.[3,15] In the small group of patients in our study, we were not able to examine this relationship. It remains to be shown whether neurotoxic tryptophan metabolites[16] or altered availability of serotonin,[3,4] or both effects together, contribute to neuropsychologic deterioration in patients. Both metabolic abnormalities, however, may result from chronic challenge of cell-mediated immunity and concomitant release of cytokines in patients with long-lasting illness such as HIV-1 infection.

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